

## Sentinel Surveillance for Zoonotic Parasites in Companion Animals in Indigenous Communities of Saskatchewan

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**Abstract.** Indigenous communities may have increased risk of exposure to zoonotic parasites, including *Echinococcus granulosus*, *Toxocara canis*, *Toxoplasma gondii*, *Dipyllobothrium* spp., and *Giardia duodenalis*, for which dogs may serve as sentinels for or sources of human infection. Canid fecal samples were collected from dogs and the environment in five indigenous communities across Saskatchewan and Alberta ( $N = 58, 62, 43, 66$ , and  $25$ ). Parasites in individual fecal samples were quantified using fecal flotation and a commercial immunofluorescent antibody test for *Giardia* and *Cryptosporidium*. Overall, the prevalence of canine intestinal parasitic infection was 20–71%, which is 5–16 times higher in indigenous communities than a nearby urban center in Saskatchewan. The overall prevalences of *T. canis*, *Dipyllobothrium*, and taeniid eggs in dog feces were, respectively, 11.8%, 4.9%, and 1.2% in our study compared with 0–0.2% in urban dogs. *Giardia* cysts present in 21% of samples were identified as zoonotic genotype Assemblage A.

### INTRODUCTION

Parasitic infections acquired by zoonotic transmission can cause serious illnesses in people and can financially burden healthcare systems.<sup>1,2</sup> In 1882, the work by Osler<sup>3</sup> published Canadian data showing that cystic hydatid disease, caused by the zoonotic parasite *Echinococcus granulosus*, was over-represented in indigenous populations compared with non-indigenous Canadians, and this trend has not changed.<sup>3–5</sup> More recently, surveillance of people residing in northern Indigenous communities across Canada has raised concerns regarding the seroprevalence of exposure to parasitic zoonoses, including *Echinococcus*, *Toxoplasma*, *Trichinella*, and *Toxocara*.<sup>2,6–11</sup>

Companion animals, such as dogs, act as bridging hosts between wildlife and people, and they can serve as sources of human infection with *Echinococcus* spp., *T. canis*, and zoonotic genotypes of *Giardia* through shedding infective parasite eggs and cysts in feces.<sup>12–14</sup> For other parasites, such as *Toxoplasma*, *Trichinella*, and *Dipyllobothrium*, dogs may serve as sentinels of shared environmental risks for humans consuming the same wild game or fish.<sup>15</sup> The widespread use of canine anthelmintics has greatly decreased the risk of dogs developing patent parasitic infections in areas where veterinary services are available; however, many northern and remote areas of Canada do not have access to these services or products.<sup>15</sup>

Currently, a knowledge gap exists in our understanding of the prevalence and significance of zoonotic parasites in people, wildlife, and domestic animals in northern and Indigenous communities in Saskatchewan. Research in other areas of northern Canada (Nunavut, northern Ontario, and Nunavik) indicates that people residing in these areas may be at higher risk of exposure to parasitic zoonoses because of a combination of unique risk factors. Large free-roaming dog populations, a reliance on locally acquired food, limited veterinary and/or medical services, and contaminated water sources are all factors that increase risk of parasite exposure.<sup>12,13,15</sup>

Past surveillance of dogs in remote Indigenous communities has identified a broad range of potentially zoonotic parasites, including nematodes (*Uncinaria* and *Trichuris*), cestodes (*Dipyllobothrium*, *Dipylidium*, and *Echinococcus*), trematodes (*Metorchis*), and protozoa (*Giardia* and *Cryptosporidium*).<sup>12,13,15–17</sup> In the 1970s, surveillance of dogs in two remote and five urban communities across Saskatchewan showed that dogs in remote areas were disproportionately parasitized compared with those dogs in urban areas of Saskatchewan. Parasites with zoonotic potential (*T. canis*, *Metorchis*, taeniids, *Dipyllobothrium*, and *Uncinaria*) had 5–52 times greater overall prevalence in canine feces from remote communities.<sup>12,16</sup> More recently, canine fecal samples collected from one Saskatchewan reserve in 2008 were 85, 153, and 8 times more likely to be infected with *T. canis*, *Giardia*, and *Cryptosporidium*, respectively, than canine fecal samples collected in Saskatoon in 2008–2009.<sup>18,19</sup> In addition, both humans and dogs on this reserve were infected with *E. granulosus*, the cause of cystic hydatid disease in people.<sup>6</sup> In the current study, we examined feces from dogs in indigenous areas of the Canadian Prairies to measure the prevalence of zoonotic parasites.

### MATERIALS AND METHODS

**Canine feces.** Between 2009 and 2011, canine feces were collected from five Indigenous rural or remote communities from public health regions in Saskatchewan (SK) and Alberta (AB; Sunrise, Mamawetan Churchill River [MCR-A and -B], and Keewatin Yatthe [KY] in SK and Chinook Health [CH] in AB) under University of Saskatchewan animal care research ethics approval 2009-0126. Fecal samples were obtained from animals (by rectal [ $N = 135$ ] or ground collection [ $N = 124$ ]) brought to mobile veterinary clinics in four communities. In three of these communities and one reserve (Sunrise), samples were simultaneously collected from the ground along major thoroughfares, on school properties, from the yards of consenting dog owners, at parks and playgrounds, and at the local landfill. Fecal samples were rejected if they appeared grey or white in color (an indicator of age of sample). All samples were sealed in labeled plastic bags and kept cool for the duration of the sampling period (1–2 days). Fecal samples were stored at  $-80^{\circ}\text{C}$  for at least 5 days to inactivate eggs of *Echinococcus* spp. Parasite eggs were quantified in

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approximately 5 g (wet weight) feces from each sample using a modified Wisconsin fecal flotation and light microscopy to identify to the family or genus level.<sup>20</sup> Approximately 1 g canine feces from each sample was screened for *Giardia* cysts and *Cryptosporidium* oocysts using a sucrose gradient flotation followed by a commercially available antibody fluorescence assay (Waterborne Inc., New Orleans, LA).<sup>21</sup> In cases where a sufficient amount of fecal matter was available for only one assay, the Wisconsin test was prioritized.

**Giardia genotyping.** Molecular methods were used to identify the genotypic assemblages of *Giardia* cysts in individual canine fecal samples from MCR-A (SK) and CH (AB) regions (number of positive samples = 15 and 21, respectively). DNA was extracted from cysts using the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA). A 511-bp segment of the  $\beta$ -giardin gene was amplified using a two-step nested polymerase chain reaction (PCR) procedure as published in the work by Lalle and others.<sup>22</sup> PCR products were resolved using ethidium bromide-stained 1.5% agarose gels, and products were visualized under ultraviolet (UV) light. PCR products were purified using the QIAquick gel extraction kit (QIAGEN Inc., Valencia, CA) before DNA sequencing with the secondary PCR primers. DNA sequencing was performed at the National Research Council Plant Biotechnology Institute (Saskatoon, SK).

**Taeniid egg speciation.** In the CH region community, taeniid eggs from canine feces were identified to species level using PCR followed by DNA sequencing. DNA was extracted from eggs using the DNeasy Blood and Tissue Kit (QIAGEN Inc., Valencia, CA). A segment of the nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 1 (NAD1) gene was amplified using primers for an approximately 500-bp region of this mitochondrial gene (JB11: 5'-AGA TTC GTA AGG GGC CTA ATA-3'; JB12: 5'-ACC ACT AAC TAA TTC ACT TTC-3').<sup>23</sup> PCR was run according to the following sequence: initial denaturation (94°C for 3 minutes), 40 amplification cycles (94°C for 15 seconds, 50°C for 30 seconds, and 72°C for 30 seconds), and final extension (72°C for 1 minute). Ethidium bromide-stained agarose gel electrophoresis was used to visualize the PCR products, which was followed by PCR product purification and DNA sequencing as described above.

## RESULTS

In the five communities sampled, 20–71% of fecal samples from client-owned dogs and/or the environment contained at

least one species of parasite, and approximately 45% of these positive samples contained multiple parasite species (Table 1). Free-roaming dogs did not have significantly higher odds of shedding parasites than client-owned dogs in MCR-A, MCR-B, or KY at the 95% confidence level, although a trend was apparent (Table 2). Overall, nematode infections were most common, with *T. canis*, *T. leonina*, and *U. stenocephala* accounting for infections in 12%, 16%, and 8% of 254 dog samples, respectively. Protozoa were also present, with *Giardia* eggs and *Cryptosporidium* oocysts identified in 21% and 4% of 231 dog samples, respectively, whereas tapeworms (taeniids [1%] and *Diphyllbothrium* [5%]) and coccidia (*Isospora* [6%]) were less common.

*Giardia* genotyping was successful in 90% (19/21) and 87% (13/15) of samples from CH and MCR-A, respectively. All were zoonotic genotype Assemblage A (GenBank accession nos. JQ978656–JQ978688). These sequences all contained a cytosine at position 606 of the  $\beta$ -giardin gene (numbered relative to *G. duodenalis* Portland I, X85958), consistent with their identification as subassemblage AI within Assemblage A.<sup>24</sup>

NAD1 sequence from taeniid eggs in a fecal sample from the CH region was similar to *Taenia pisiformis* (88% identical over 491 nucleotides to AJ239109; GenBank accession no. JQ917875). The remaining sample had a low egg count (5 eggs/g) and did not amplify on PCR.

## DISCUSSION

**Sources and sentinels.** Our study shows that dogs in remote and rural areas can act as both sources and sentinels for human exposure to zoonotic parasites.<sup>15,25</sup> Parasites of known public health concern were found in all communities. For some of these parasites, pets are a potential source of human exposure. For example, people can become accidental hosts for larvae of the roundworm *Toxocara* spp. when they ingest eggs passed in pet feces or possibly, larvae encysted in paratenic hosts.<sup>25</sup> Toxocariasis can cause ocular and visceral larval migrans, and it is the most frequent parasitic zoonoses passed from pets to people in the United States.<sup>12,26–30</sup> Clinical toxocariasis may be less common in Canada, with toddlers at highest risk.<sup>31</sup> Dog ownership was not an important risk factor for seropositivity for *T. canis* in Canada, emphasizing the importance of environmental contamination by free-ranging dogs for transmission of this zoonosis.<sup>32,33</sup> Canids, including domestic dogs, are the definitive host for tapeworms in the Taeniidae family, and they pass eggs infective to people in their

TABLE 1

Prevalence of intestinal parasite eggs, cysts, and oocysts in canine feces collected in indigenous communities across Alberta and Saskatchewan public health regions as identified through quantitative sucrose flotation and immunofluorescent assay

	Chinook Health	Mamawetan Churchill River A	Mamawetan Churchill River B	Sunrise	Keewatin Yatthe
Community ID	CH	MCR-A	MCR-B	SR	KY
Month/year	9/2009	9/2011	6/2010	11/2011	5/2010
<i>Toxocara</i>	9/62 (15%)	9/58 (16%)	7/66 (11%)	2/25 (8%)	2/43 (5%)
<i>Toxascaris</i>	25/62 (40%)	8/58 (14%)	3/66 (5%)	1/25 (4%)	3/43 (7%)
<i>Uncinaria</i>	0/62 (0%)	20/58 (34%)	0/66 (0%)	0/25 (0%)	0/43 (0%)
Taeniid	2/62 (3%)	0/58 (0%)	1/66 (2%)	0/25 (0%)	0/43 (0%)
<i>Diphyllbothrium</i>	0/62 (0%)	10/58 (17%)	1/66 (2%)	0/25 (0%)	1/43 (2%)
<i>Isospora</i>	3/62 (5%)	2/58 (3%)	3/66 (5%)	0/25 (0%)	7/43 (5%)
<i>Giardia</i>	13/40 (22%)	21/57 (37%)	5/66 (8%)	1/25 (4%)	0/43 (0%)
<i>Cryptosporidium</i>	4/40 (10%)	1/57 (2%)	1/66 (2%)	1/25 (4%)	1/43 (2%)
Overall*	38/62 (61%)	41/58 (71%)	15/66 (23%)	5/25 (20%)	11/43 (26%)

\*Overall prevalence measured as the number of samples containing at least one parasite species divided by the total number of samples.

TABLE 2

Comparison of parasite prevalence in feces collected from dogs brought to remote animal health clinics (client-owned dogs) versus feces collected off the ground (environmental) in three indigenous Saskatchewan communities

Public health region sampling site	Client-owned dogs	Environmental	Odds ratio	95% Confidence interval
MCR-A	25/40 (63%)	16/18 (89%)	4.8	1.0–23.9
MCR-B	4/16 (25%)	11/55 (20%)	1.0	0.3–3.7
KY	3/17 (18%)	8/26 (31%)	2.1	0.5–9.3

feces. At the microscopic level, all taeniid eggs appear alike, and molecular techniques are needed to identify species.<sup>6</sup> Although we found non-zoonotic *T. pisiformis* in southern Alberta, other species, including *T. crassiceps* and *E. granulosus*, are potentially present, and mixed infections would not necessarily have been detected by the techniques that we used.

Dogs may be a source of human infection, but they are also potential recipients of infection from human sewage.<sup>34</sup> *G. duodenalis* has been identified in a variety of wildlife species and companion animals in Saskatchewan, including dogs, coyotes, and beavers.<sup>18,19,35,36</sup> It is most often spread by direct contact or contaminated food and water sources.<sup>35</sup> In our study, 2% to 37% of canid fecal samples from four communities were positive for *G. duodenalis*, and the zoonotic genotype A was confirmed in dogs in two communities. The work by Himsworth and others<sup>18</sup> found a prevalence of 61% in dog feces from another indigenous community in SK, whereas the work by Gaunt and Carr<sup>19</sup> found a prevalence of 0.4% in dogs from an urban center in SK. However, prevalence of *Giardia* is generally underestimated in surveillance studies because of the sporadic shedding of cysts, poor sensitivity of flotation assays, and potential for subclinical infection.<sup>31</sup> *G. duodenalis* Assemblage A has been identified in dogs in SK, AB, and the Northwest Territories, and it is considered zoonotic because of its lack of host specificity (hosts include dogs, cats, and people).<sup>15,18,37</sup> Assemblage A is known to be more virulent in people than other genotypes, and it should be considered a potential risk to public health in the CH and MCR regions where we identified infection in dogs.<sup>35,38</sup>

For other parasites, dogs are not direct sources of human infection, but instead, they serve as sentinels for parasites that are acquired by both people and dogs through common routes of exposure. For example, cestodes in the genus *Dipyllobothrium* (most likely *D. latum* or *D. dendriticum*) cannot transmit directly from dogs to people. Instead, both are infected through the consumption of infective plerocercoids in raw or undercooked fish.<sup>38</sup> Dogs are at least as susceptible as people, and infection can be detected through relatively non-invasive fecal diagnostic tests. Dogs may also serve to amplify the abundance of this cestode in the environment. For animal sentinels to be useful for public health purposes, they must be highly susceptible and highly exposed, and they must show a detectable response. Because dogs in remote and Indigenous communities are often free-ranging and have access to human food and garbage and carcasses of local fish and wildlife, they are highly exposed and thus, make excellent sentinels.

**Disparities and community-specific parasite profiles.** Dogs in Indigenous and northern communities in western Canada had a markedly higher prevalence of parasite shedding than dogs in a nearby urban center.<sup>19</sup> This finding is most likely attributable to factors such as age, reproductive status, gender, housing, geographic region, diet, and access to veterinary

care.<sup>14,39</sup> For example, dogs brought to mobile veterinary clinics in Indigenous communities are quite young (mean age = 1–2 years), likely because older animals have already been sterilized. Free-ranging dogs in these communities are also young, possibly as a result of dog management practices (e.g., dog shoot days) and high natural mortality in many communities. Juvenile dogs are more likely to shed parasites, which may, in part, account for the high prevalence that we observed.

Our study revealed a distinct profile of parasite shedding and exposure in each community, even in those communities in relatively close proximity; this finding is likely the result of variation in risk factors such as access to harvested wildlife, human garbage, clean water, and veterinary services.<sup>29</sup> However, it is also important to note that parasite shedding is affected by season, which varied among the sample collections. One possible explanation for the low prevalence of *Uncinaria* infection was freezing at –80°C, which may have rendered the eggs unidentifiable. As well, some fecal samples collected from the ground may have originated from the same animal, causing the population prevalence to be over- or underestimated, depending on whether the animal was shedding parasite eggs. Sample collectors were unable to distinguish canine feces from the feces of wild canids, such as wolves or coyotes; however, these wildlife are considered unlikely within the communities.

Identifying local risk factors and developing community-specific parasite profiles can significantly aid veterinarians and health professionals in introducing locally effective animal and human health interventions. Key messaging in knowledge translation includes administering a broad-spectrum dewormer to companion animals regularly (at least one time per year), removing and disposing of animal waste regularly, cooking meat and fish consumed by both people and pets, and washing hands before eating and after handling animals or animal waste.<sup>29</sup> Population control of free-roaming dogs and preventative healthcare for owned dogs are crucial components to decreasing environmental contamination; many parasite eggs and cysts can survive months to years in the environment and are resistant to commonly available disinfectants. This resistance will require improved access to veterinary products and services that are currently unavailable in the entire northern one-half of SK. Finally, our work suggests that surveillance of parasites in companion animals is a potential tool for detection of zoonotic risks for people, and it could be used to evaluate the efficacy of animal and public health interventions. Using sentinels in this way could benefit communities by producing rapid, discrete, and economical estimates of human health risk and simultaneously improving both animal and public health. Additional investigation into this application for animal sentinels requires exploring the relationship of prevalence levels and parasite species between people and companion animals.



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